

Figure 1:

Amino acid sequences of Cpn60 and Cpn10:

SEQ ID No 1: Cpn10 (encoded by nucleotides pos. 458-751 of Figure 2):

MKIRPLHDRVVRRKEEETATAGGIILPGAAAEKPNQGVVISVGTGRILDNGSVQALA
VNEGDVVVFGKYSGQNTIDIDGEELLILNESDIYGVLEA

SEQ ID No 2: Cpn60 (encoded by nucleotides pos. 800-2446 of Figure 2):

MAAKDVLFGDSARAKMLVGVNIIADAVRVTLGPKGRNVVIEKSFGAPIITKDGVSV
AREIELKDKFENMGAQMVKEVASQANDQAGDGTATVLAQAIISEGLKSVAAGMN
PMDLKRGIKATAAVVAAIKEQAQPCLDTKAIAQVGTISANADETVGRLIAEAMEKV
GKEGVITVEEGKGLEDEL DVVEGMQFDRGYLSPYFINNQEKM TVEMENPLILLVDKK
IDNLQELLPILENVAKSGRPLLIVAEDVEGQALATLVVNNLRGTFKVA AVKAPGFGD
RRKAMLQDLA ILTGQQVISEELGMSLETADPSSLGTASKVVIDKENTVIVDGAGTEAS
VNTRVDQIRAEIESSTSDYDIEKLQERVAKLAGGVAVIKVGAGSEMEMKEKKDRVD
DALHATRAAVEEGVVAGGGVALIRALSSVTVVGDNEDQNVGIALALRAMEAPIRQI
AGNAGAEGSVVVDKVKSGTGSFGFNASTGEYGDMIAMGILDPK VTRSSLQAAASI
AGLMITTEAMVADAPVEEGAGGMPDMGGMGGMGGMGPOMM

[illegible]

Figure 2 (continued):

cacaaccgaagccatgggtgcggatgcgcctgttgaagaaggcgctgggtgatgcctgatagggcggcattgggtggaatgggcg
gtatgcctggcatgatgtaatcactttgtgattcattgtcctgatctgctaccgtgtaaaaagatcaggctcaaggctgtctataaaaag
ccgtatcttgatgagtgtgtcttctgtgaaaacgacattctggagtgcggccttttttgatttggtcataaaattcagaatatgtgtaatt
ttatgtaactagctggcctataaagtgtgagttcctctgggtggcatgatctcatggtaacttaagcctgattcactgcg
gccttaacagtaaaataataacgcaacgtagaacaataaagcgtatggcataaataagacggctgcatttaattcagatc

Figure 3:

SEQ ID No 4: Amino acid sequence of esterase cloned from *Oleispira antarctica* (EstRB8):

EstRB8 (encoded by nucleotides 1145 to 2143 Frame 2 of Figure 4) 333 aa

MKNTLKSSSRFSLKQLGTGALHSSLFFGGCTTTQQDNLYTGVM SLARDSAGLEVKTA
SAGDVNLT YMERQGS DKN A ESVILLHGFSADKDNWILFTKEFDEKYHVIAVDLAG
HGDSEQLLT TDYGLIKQAERLDIFLSGLGVNSFHIAGNSMGG AISAIYSLSHPEKV KSL
TLIDAAGVDGDTESEYYKVLAEGKNPLIATDEASFEYRMGFTMTQPPFLPWPLRPSLL
RKTLARAEINN KIFSDMLKTKERLGMTNFQQKIEVKMAQHPLPTLIMWGKEDRVLD
VSAAA AFKKIIPQATVHIFPEVGH LPMVEIPSESAKVYEEFLSSIK

Figure 4:

SEQ ID No 5: DNA fragment from plasmid pBK1Est coding for esterase of *Oleispira antarctica* (EstRB8):

Nucleotide positions 1-100 correspond to reverse complement of positions 1196-1121 and 3799-3939 correspond to reverse complement of 1043-952 of pBK-CMV vector (Stratagene).

Positions 101-105 are *Bam*HI – *Sau*3A1 fusion and positions 3795-3798 are *Sau*3A1-*Bam*HI-fusion.

acaggaaacagctatgaccttgattacgccaagctcgaaattaacccctactaaagggacaaaagctggagctcgcgccctgcag
gtcgacactagtggaalcaacggcggttcattggtactggctgagttcagcgicataatgccgatggcgaactggccgtcactgagtagt
tcttctgtagcaccgatttttctaatagcgcagcctcttttctgaacgggcaactgatgtagtttttactaaccggccttttaggcattg
taaactcttcgatattcaaaattattactgttcataattacaatcatagtagcaggctagaggcccaaaattgcagctgatattcacccttatttc
taagcattattacactcatcgcggtgtttatttaaitgtgctaaataaaataaccgtagcggaanaattcagcaaatagccaaagaaaacga
ttggcaataaccaagaattcagctgtttgatgatgacattagcaggcaaaccttggcctattaaactacagtcaaaatgcaatttttagacat
ctcattcaagcaactgacgaacactatggcttagcggttaagacccttgacitgtagcggttagaaccctcaggtattcacaatagcagctt
tattttatttaccctcgactaaagactgaattcaataacctacacatttgcttaagtcgacatattcaagataaagatgccctcactgacatca
gtcaccacaatacaatcaaacaccaataccaatcgcaaaaactcataaaactagcgcatcaccaaatcccaaaaggcgttcaaaaatgaa
acgagcacgctcacacaaaatcaattatagctaacgaaccagggtcaaacctatcgctttttttagcagcgtttgtccactaatgaaagaga
aaagtcgttaattcactggcctttggcgtaiccgcccttcacatagaaatagtaattggcatgctactggccttfaaaagaatcagtttaatt
gaagaaaccicgcttattcagccattaccgctgtagcgaatttgcgcttaccctcagccatgaltaaactgacgccaattatataagac
atactaataataactcccttaattgagaagaataatgaaaaacacactcaaatctcattacggttttagctgaaacaactcggcaccggc
gctctgattatctcaggtttgttcttgggtgggtgcaccacaacacaacagataatttatacacagggttatgtctcttcgagagacagc
gctggcctagaagftaaaacagccctcgccgggtgacgtcaatcttactatattggaacgccaaggcagtgacaaagataatgccgaag
cggtattttatcacgggttctctgctgataaagataactggattcttttaccaaagaattcgatgaaaatatcatgtatcgtctgcattta
gggggacatggcgattcagaacaatttatiaacgactgattacgggtctcataaaacaagccgagcggttagatactcttcttctggcctagg
gggttaactcatttcacatcgccggttaattcaatggggggggctatcagcgcaactctacagtttgagtcacccagagaaaagttaaaagtctt
acattgatcgatgcagcagggtgctgatggcgatctgaaagcgaatactacaaagttttggcagaaggtaagaatcctttaattgcaact
gatgaageaagtttgaataccgcaagggtttaccatgactcagcctcttcttacccttggccactaagacccttattatcgttaaaacg
ctagcccggtccgagatcaataacaaaatttttccgatatgctgaaaaccaaagaacgcttaggaatgactaaccttcaacagaaaattg
aagtgaanaatggctcaacatccattgccaacctgattatgtggggcagaagaagatcgcttcttgacgtatccgcagcagcgcccttc
aaaaaataatccacaagcaactgttcataatttctctgaagtaggccacctacctatggtagaaattccatgtgaagcgctaaagttat

Figure 4 (continued):

gaagagfittgtctctattaaataagagcacataatcatgactgacttataaacagccaagcatttaaagtctggctgtttatttfaatgg
ccaaattattcaacgaccaagctcgcggtaaaatcgagtggtttctgttttcaacaacagcaacaacgtgaaatccccgtaateg
cattttctgattatcaaaatacactttccaccagcatatttaacttaacttttaactctgcccctacctctataacactggcagtcgaatt
cgacaatggfaccctgcgggaacaggatgcttaaaatcgatcgatcactgctgacgggtacgatgctttgtcgagaaaaacgagtcgct
gcaataaaagaaacccatccactgcatgagtgccaccgaataacgtaicagatgattgtgtctcgtgaaataccgctttaga
aafagtggfittgatacgcgcttctgctgcgaataatctctctgctaagagttggggaaggcatacafaaaatcgcttgattaagatta
ataataaatagtaacagtalattgaacigagggctggaagaacitaaacacctgaagaactttgagggccgctagagagaaaagacca
gtgataataittcatcttgccatgagagcttatcatgaagcctctgtcttaaaatcaatcattatatttattcatctttaattgaaataaaccaat
atatctcatataatttcacactaccttatctcactagacttcccgcgcataggcgcaacaatcaacgcaagttcacaataaagcgggtc
gctgcaacacatgccctagcgcttaagtagcacgcacaacactggccagtcgactagcccttgcgattcgtgcagacgagcaac
aagegctatfaaacttaactaaattctaaaccaccacatgggttctttccaaaactcaaaaaacgcgcaaatccgcttgcaatttaaagc
cgatgacatagatctatcgattatcaaacccgcattcaagcgtcatlaaaaacgcaccacitggcaagaagttctacctgcactgacca
atatgcaagcggcggcggaagagctgctttgatcgatcaagaaaggggagcagcaagaggaaaaaatcaaaaaggagga
gcaatcaataaaaaacgagttatigaggatttattttaaacaagggtatataataacctctctcgtagtaacaatgactgtatttacaaa
aaataaalagaggatatacattgcaaacatctgggttgaagtaaccaagattgaagiatlaaacgctcaaatggaaaaactgctgcagc
aaacttaggcattcaattacagaaattggcgatgattatatactggcacaatgccagcagatgcacgtacctccagcaatgggactg
attcatggcggctcaaatgtattgctggcagaaacactgggcagcaltggcagctaactgctgtattatgtctcaagaatattgtgtgg
ccaagaaattaacgccaaccacatcgcggtgtcgttccggcatagtactggcacagcaacgctagtaacaaaggaagaacctc
ccagatttgggaaattcgcatcgtaacgatccaaagaattcaaaaagcttctcgagagtactttagagcggcggggcccatcgati
ttccaccgggtggggtaaccaggtaaggttaaccaattcgccctatagttagtctgattacaattcactggcgtgtgtttac

Figure 5:

Amino acid sequences expressed from vector pBK1CpnEst: - the co-expression of fragments encoding native chaperonins with the esterase gene (EstRB8), all from *Oleispira antarctica*

SEQ ID No 6: cpn10 (nucleotides 113 to 403; Frame 2 of Figure 6) 97 aa:

MKIRPLHDRVVRRKEEETATAGGHLPGAAAEKPNQGVVISVGTGRILDNGSVQALA
VNEG DVVVF GKYS GQNTIDIDGEELLILNESDIYGVLEA

SEQ ID No 7: cpn60 (nucleotides 455 to 2098; Frame 2 of Figure 6) 548 aa:

MAAKDVLFGDSARAKMLVGVN ILADAVRVT LGPKGRNVVIEKSFGAPIITKDGVSV
AREIELKDKFENMGAQM VKEVASQANDQAGD GTTTATVLAQAIHSEGLKSVAAGMN
PMDLKR GIDKATAAVVAAIKEQAQPCLDTKAIAQVGTISANADETVGR LIAEAMEKV
GKEGVITVEEGKGLEDEL DVVEGMQFDRGYLSPYFINNQE KMTVEMENPLILLVDKK
IDNLQELLPILENVAKSGRPLLIVAEDVEGQALATLVVNNLRGTFKVA AVKAPGFGD
RRKAMLQDLAILTGGQVISEELGMSLETADPSSLG TASKVVIDKENTVIVDGAGTEAS
VNTRVDQIRAEIESSTSDYDIEKLQERVAKLAGGVAVIKVGAGSEMEMKEKKDRVD
DALHATRAAVEEGV VAGGVALIRALSSVT VVGDNEDQNVGIALALRAMEAPIRQI
AGNAGAEGSVVVDKVKSGTGSFGFNASTGEYGD MIAMGILDPAKVTRSSLQAAASI
AGLMITTEAMVADAPVEEGAGGMPDMGGMGGMGGMGMPGMM

SEQ ID No 8: estRB8 (nucleotides 2579 to 3577; Frame 2 of Figure 6) 333 aa:

MKNTLKSSSRFSLKQLGTGALIHSSLFFGGCTTTQQDNLYTGVM SLARDSAGLEVKTA
SAGDVNLFYMERQGS DKNDAESVILLHGFSADKDNWILFTKEFDEKYHVI AVDLAG
HGDSEQLLT TDYGLIKQAERLDIFLSGLGVNSFH IAGNSMGGAISAIYSLSHPEKV KSL
TLIDAAGVDGDTESEYYKVLAEGKNPLIATDEASFEYRMGFTMTQPPFLPWPLRPSLL
RKTLARAEINN KIFSDMLKTKERLGMTNFQQKIEVKMAQHPLPTLIMWGKEDRVLD
VSAAAAFKKIIPQATVHIFPEVGHLP MVEIPSES AKVYEEFLSSIK

Figure 6:

SEQ ID No 9: pBK1CpnEst: - the fusion of native chaperonine-coding fragments with
esterase of *Oleispira antarctica* (EstRB8)

The DNA fragment coding for Cpn10 and Cpn60 is flanked by *SacI* site (pos. 69-75) and *SalI*
site (encoded by pos. 2138-2143 of Figure 7):

Nucleotide positions 1-75 correspond to reverse complement of positions 1196-1121 and
positions 5233-5273 correspond to reverse complement of 1043-952 of pBK-CMV vector
(Stratagene)

Small letters -- the Cpn10-Cpn60 encoding fragment,

Capital italics -- fragments of vector pBK-CMV

Capital letters -- fragment coding for EstRB8 from plasmid pBK1Est

ACAGGAAACAGCTATGACCTTGATTACGCCAAGCTCGAAATTAACCCCTCACTAAAGGGA
ACAAAAGCTGGAGCTCtaataacttgggatccaacagttggagagctagcaaatgaaaatccgtccattacatgatcgtatt
gttcttcgccgtaaagaagaagagaccgcaactgcgggttggtattattaccgggcgtgcggcagaaaaaccaatcaaggtgtgt
tatctcgtgggtacttgcgtattcttgataatgggtcagtgcaagcgtggcggtaacgaaggcgatgtgtcgttttggtaaatactc
aggtcaaaatactatcgatcgtatgggtgaagaattatgatttgaaatgaaagtatactacgtttagaagcttaattattacactca
ctttttatitaacctacaaaatttaaggaaagatcgtgcttaagacgtatatttgggtgagcgcacgcgcaaaaatgttggtaggt
gtaaacattttagcgcagcagtaagagttaccttaggacctaaaggctgtaacgttgtatagaaaaatcatttgggtgcaccgatcatcac
caaagatggtgttctgttgcgcgtgaaatcgaaatgaaagacaaatcgaaaacatggcgccacagatggttaaggaaagtgtcttca
agccaacgaccaagccggtgacggcacaacgacagcgtactagcacaggcgattatcagcgaaggctgaaaatcgttgcgg
ctggcatgaatccaatggatctaaacgtggtatlgataaagctacggctgctgtgttgcgcctaaagaacaagctcagccttgcctg
gatacaaaagcaatcgtcaggtagggacaactctgccaatgccgaagaacgggtggcgttaatttctgaagcgaatggaaaaagt
cggtaagaagggtgtgattaccgtgaagaaggcaaggcctgaagacgagcttgatgtttagaaggcatgcagctcgaatcgcgggt
actgtctccgtactcatcaacaaccaagaaaaaatgaccgtagaatggaaaatccatattctattggtgataagaaaattgataac
cttcaagagcgttgcgaattctgaaaacgtcgttaaatcaggctcgtccattattgatcgttgcgtgaagatgttgaaggccaagcactagc
aacattggtagtaacaacttgcgcggcacatcaagggttcagcgggttaagccccctgggttggcgatcgtcgtaaagcagtggtgca
agatcttgcacatctgacgggtggcaggttattctgaagagcagggatgtcttagaaactgcggatccttcttcttgggtacggcaa
gcaagggtgttatcgataaagaaaacaccgtgatgttgatggcgaggtactgaagcaagcgttaatactcgtgttgaccagatccgtg
ctgaaatcgaaaagctcgacttctgattacgacatcgaaaagttacaagaacgcgttgcctaaagcttgcggcgccgttgcgtgattaaag

Figure 6 (continued):

[illegible]

Figure 6 (continued):

CCTATGGTAGAAATTCCTAGTGAAAGCGCTAAAGTTTATGAAGAGTTTTTGTCTCT
CTATTAAATAAGAGCACATAATCATGACTGACTTATAAACAGCCAAGCATTAAAA
ATGCTTGGCTGTTTATTTTAATGGCCAAATTATTCAACGACCAAGCTCTGCGGTAA
AATCGCAGTGGGTTTCTTGTTCATCAACAGCAACAAACGTGAAATACCCCGTA
ATCGCATTTTTCTGATTATCAAAATACATACTTTCCACCAGCATATTAACCTCAAC
TTTTAAACTCGTCCGCCCTACCTCTATAACACTGGCAGTCAATTTCGACAATGGTAC
CTGCGGGAACAGGATGCTTAAAATCGATTTCGATCACTGCTGACGGTTACGATGCT
TTGTGAGAGAAAAACGAGTCGCTGCAATAAAAGAAACCTCATCCATCCACTGCATT
GCAGTGCCACCGAATAACGTATCATGATGATTGTTGTCTCTGGAAATACCGCTTT
AGAAATAGTGGTTTTTGATACGCGCTTTCGCTGCGCAATAATATCTTCTCTGCTAA
GAGTTGCGGATGGCATACTAACTCGCTTGATTAAGATTAATAATAAATAGTTA
ACAGTATATTGAACTGAGGGTCTGAAGAACTCTAATACCTCTGAAGAACTTTGAG
GCCGCTAGAGAGAAAAGACCAGTGATAATATTTTCATCTTGCCATGAGAGCTTATC
ATGAAAGCCTGTGCTTAAAATCAATCATTATATTTATTCATCTTTAATTGAAATAA
TACCAATATATTTTCATATATAATTTTACACTACCTTATCTCACTAGACTTCCCGC
GCATAGGCGCAAACAATCAACGCAAGTTCACAATAAAGCGGTTTCGCTGCAACAC
ATGCCCTAGCGTCTAAAGTAGCACGCACAACACTGGCCAGTCGTACTAGCCCTT
TGCGATTTCGTGCAGACGAGCAACAAGCGCTATTAAACTTACCTAAATTTCTAACC
ACCACCATTTGGTTCTTTTCCACAACTCAAAAACTCGTCAAATCCGCTTGCAATT
TAAACGCGATGACATAGATCTAATCGATTATCAAACCCGCATTCAAGCGCTCATT
AAAAACGCACCACTGGCAAGAAGTTCTACCTGCACTGACCAATATGCAAGCGGC
GGCGGAAGAGCTGCCTTTGATCGATCAAGAAGAAGGGAGCAGCAAAGAGGAAA
ACAATCAAAAAGAGGAGAGCAATCAAATAAAAAACGAGTTATTGAGGATTTTAAT
TTTAAACAGGTATATTAATACCCTCTCTCGTAGTAAACAATGACTGTATTTACAC
AAAAATAAATAGAGGTATACCATGTCAAACATCTGGTTTGAAGTACCAAAGATTG
AAGTATTAAACCGTCAAATGGAAAATACTGCCTGCAGCAACTTAGGCATTCAAAT
TACAGAAATTGGCGATGATTATCACTGGCACAATGCCAGCAGATGCACGTACC
TTCCAGCCAATGGGACTGATTCATGGCGGCTCAAATGTATTGCTGGCAGAAACAC
TGGGCAGCATGGCAGCTAACTGCTGTATTAATTTGTCTCAAGAATATTGTGTTGG
CCAAGAAATTAACGCCAACCACATACGCGGTGTTTCGTTCCGGCATAGTGACTGGC
ACAGCAACGCTAGTACACAAAGGAAGAACCTCCCAGATTGGGAAATTCGCATC

Figure 6 (continued):

GTTAACGATCCAAAGAATTCAAAAAGCTTCTCGAGAGTACTTCTAGAGCGGCCGCGGG
CCCATCGAATTTCCACCCGGGTGGGGTACCAGGTAAGTGTACCCAATTCGCCCTATAGT
GAGTCGTATTACAATTCACCTGGCCGTCGTITTAC

Figure 7:

Amino acid sequences expressed from vector pBK1CpnSREst: - the co-expression of the stabilized single ring mutant chaperonin with the esterase gene (EstRB8) from *Oleispira antarctica* (cpn10::stabilized single ring mutant Glu460Ala/Ser462Ala/Val463Ala::est)

SEQ ID No 10: cpn10 (nucleotides 113 to 403; Frame 2 of Figure 8) 97 aa:

MKIRPLHDRIVVRRKEEETATAGGIIIPGAAAEKPNQGVVISVGTGRILDNGSVQALA
VNEG DVVVF GKYSQNTIDIDGEELLILNESDIYGVLEA

Below – **Capital bold letters** are the mutations introduced

SEQ ID No 11: stabilized single ring mutant of cpn60 (nucleotides 455 to 2098; Frame 2 of Figure 8) 548 aa:

MAAKDVLFGDSARAKMLVGVNILADAVRVTLGPKGRNVVIEKSFGAPIITKDGVS
AREIELKDKFENMGAQMVKEVASQANDQAGDGT TATVLAQAIISEGLKSVAAGMN
PMDLKR GIDKATAAVVAAIKEQAQPCLDTKAIAQVGTISANA DETVGR LIAEAMEKV
GKEGVITVEEGKGLEDEL DVVEGMQFDRGYLSPYFINNQE KMTVEMENPLILLVDKK
IDNLQELLPILENVAKSGRPLLIVAEDVEGQALATLVVNNLRGTFKVA AVKAPGFGD
RRKAM LQDLA ILTG GQVISEELGMSLETADPSSLGTASKVVIDKENTVIVDGAGTEAS
VNTRVDQIRAEIESSTSDYDIEKLQERVAKLAGGVAVIKVGAGSEMEMKEKKDRVD
DALHATRAAVEEGVVAGGGVALIRALSSVTVVGDNEDQNVGIALALRAMEAPIRQI
AGNAGAAGAAVVDKVKSGTGSFGFNASTGEYGDMIAMGILDPK VTRSSLQAAASI
AGLMITTEAMVADAPVEEGAGGMPDMMGGMGGMGGMPGMM

SEQ ID No 12: EstRB8 (nucleotides 2579 to 3577; Frame 2 of Figure 8) 333 aa:

MKNTLKSSSRFSLKQLGTGALHSSLF FGGCTTTQQDNLYTGVM SLARDSAGLEVKTA
SAGDVNLT YMERQGS DKN AESVILLHGFSADKDNWILFTKEFDEKYHVIAVDLAG
HGDSEQLLT TDYGLIKQAERLDIFLSGLGVNSFHIAGNSMGG AISAIYSLSHPEKV KSL

Figure 7 (continued):

TLIDAAGVDGDTSEYYKVLAEGKNPLIATDEASFEYRMGFTMTQPPFLPWPLRPSLL
RKTLARAEINNKFSDMLKTKERLGMTNFQQKIEVKMAQHPLPTLIMWGKEDRVLD
VSAAAFAFKIIPQATVHIFPEVGHLPMVEIPSESAKVYEEFLSSIK

Figure 8:

SEQ ID No 13: DNA sequence of vector pBK1CpnSREst: the expression cassette for the co-expression of the stabilized single ring mutant chaperonin with the esterase gene (EstRB8) from *Oleispira antarctica* (cpn10::stabilized single ring mutant Glu460Ala/Ser462Ala/Val463Ala::est)

Nucleotide positions 1-75 correspond to reverse complement of positions 1196-1121 and positions 5233-5273 correspond to reverse complement of 1043-952 of pBK-CMV vector (Stratagene)

DNA fragment coding for Cpn10 and Cpn60 is flanked by *SacI* site (pos. 69-75) and *Sall* site (pos. 2138-2143).

In the DNA sequence:

Small letters --- the Cpn10-Cpn60 coding fragment,

Capital italics -- fragments of vector

Capital letters -- fragment coding for EstRB8 from plasmid pBK1Est

Capital bold letters = introduced mutations

ACAGGAAACAGCTATGACCTTGATTACGCCAAGCTCGAAATTAACCTCACTAAAGGGA
ACAAAAGCTGGAGCTCetaatacttgggatccaacagttggagagtctagcaaatgaaaatcgttcattacatgatcgtt
gttgtegcgtaaaagaagaagagaccgcaactgcgggtggtatttatttaccggcgctgcggcagaaaaaccaaataaggigtgt
taletctgtgggtactggccgtattctgataatgggtcagtgcgaagcgtggcggttaacgaaggcgatgttgcgttttggtaatactc
aggtaaaatactatcgatcgtggtgaagaattatgatttgaatgaaagtgaatctacggcggtttagaagcttaattattacactca
ctttttatttaacctacaaaatttaaggaaagatcatggctgctaaagacgtatttgggtatagcgcacgcgcaaaaatgttggtaggt
gtaaacattttagccgacgcagtaagagttaccitaggacctaaaggcgttaacgttattatagaaaaatcatttgggtgcaccgatcatcac
caaagatgggtgtttctgttgcgctgaaatcgaaatgaaagacaaattcgaaaacatgggcgcacagatggttaagggaagttccttca
agccaacgaccaagccggtgacggcacaacgacagcgactgtactagcacaggcgattatcagcgaaggcttgaatctgttgcgg
ctggcatgaatccaatggatcttaaacgtggtaattgataaagctacggcgtgtgtgttgcgccattaaagaacaagctcagccitgcttg
gatacaaaagcaatcgctcagglaggacaaatctctgccaaatgccgatgaacgggtgggtgttattgtctgaagcgatggaaaaagt
cggtaaaagaggtgtgattaccgttgaagaaggcaaggccttgaagacgagctgtatgtttagaaggcatgcagttcgatcgcgggt
actgtctccgtacttcatcaacaaccaagaaaaaatgaccgtagaaatggaaaaatcatttaattctattggttgaagaaaattgataac
cttcaagagctgttgccaattctgaaaacgttcgctaaatcaggtcgtccattatgatcgttgcgtgaagatgttgaaggccaagcactagc

Figure 8 (continued):

aacattgtagtaaacacttgcgcggcacattcaagggtgcagcgggtaagcccctgggttggcgatcgctglaaagcgatgttgca
agatcttccalcitgacgggtggcagggtatttctgaagagctagggatgtcttagaaaactgcggatccttcttcttgggtacggcaa
gcaagggtgtatcgataaagaaaacaccgtgattgttgatggcgagggtactgaagcaagcgttaatactegttgacagatccgtg
ctgaaatcgaaagctcgacttctgallacgacatcgaaaagttacaagaacgcgttgctaaagcttgcggcgccggtgcccgtgattaag
gttgggtgcgggttctgaaatggaatgaagagaagaaagaccgtgttgacgatgcacttcacgaactgcgcgcagcgggtgaagaag
gtgtgttgcgggtggtggtgtgtcttggatcgcgcaactctctcagtaaccgtgtgtggtgatnacgaagatcaaacgtcggtattgcat
tggaacttgcgtcgatggaagctcctalcgcgtaaacgcgggtaacgcagggtctgCagggGcagCgggtgtgataaagtgaal
ctggcacaggtagcttgggttaacgccagcacagggtgagtatggcgatatgattgcgatgggtatttagacctgcaaaagtcacgc
gttcactctacaagccgcggcgctctatcgagggttgatgatcacaaccgaagccatgggtgcggatgcgcctgttgaagaaggcgct
gggtggtatgcctgatatggggcgcatgggtggaatggggcggtalgcctggcgatgatgaatcacttgtgattcattgtctctgattgctta
ccgtGTTCGACATATTCAAGATAAAGATGCCTTCACTGACATCAGTCACCAACAATC
AATCAAACACCAATACCAATCGCAAAAACCTCATAAACTAGCCGATCACCAAAAT
CCCAAAAGCGTTCAAAAATGAAACGAGCACGTCACACAAAATCAATTTATACGC
TAACGAACCAGGTCAAACCTTATCGTTTTTTTGAGCACGTTTGTTCCTACTAATGAAA
GAGAAAAGTCGTTAATTCAGTGGCTTTTGGCGTATCCGCACCTTCACATAGAAAT
TAGTAATGGCATGCTACTGGCCTTTAAAAAGAATCAGTTAATTGAAGAAACCTCG
CTTATCTCAGCCATTACCGCTGTAGCCGAATTTGCGCTTATCCTCAGCCATGATTA
AACTGACGCCAATTAATATAAGACATACTAATTAATAACTCCCTTAATTGAGAAG
AATAATGAAAAACACACTCAAATCCTCATCACGTTTTAGTCTGAAACAACCTCGGC
ACCGGCGCTCTGATTATCTCCAGTTTTGTTCTTCGGTGGTTGCACCACAACACAACA
AGATAATTTATACACAGGGGTTATGTCTCTTGGGAGAGACAGCGCTGGCCTAGAA
GTTAAACAGCCTCTGCCGGTGACGTCAATCTTACTTATATGGAACGCCAAGGCA
GTGACAAAGATAATGCCGAAAGCGTTATTTTATTACACGGTTTCTCTGCTGATAA
AGATAACTGGATTCTTTTTACCAAAGAATTCGATGAAAAATATCATGTTATCGCT
GTCGATTTAGCGGGACATGGCGATTGAGAACAAATFATTAACGACTGATTACGGTC
TCATAAAACAAGCCGAGCGTTTAGATATCTTCTTATCTGGCTTAGGGGTTAACTC
ATTCACATCGCCGGTAATTCAATGGGGGGGGCTATCAGCGCAATCTACAGTTTG
AGTCACCCAGAGAAAGTTAAAAGTCTTACATTGATCGATGCAGCAGGIGTCGATG
GCGATACTGAAAGCGAATACTACAAAGTTTTGGCAGAAGGTAAGAATCCTTTAAT
TGCAACTGATGAAGCAAGTTTTGAATAACCGCATGGGTTTCACCATGACTCAGCCT
CCTTTCTACCTTGGCCACTAAGACCTTCTTTATTACGTAAAACGCTAGCCCGTGC
CGAGATCAATAACAAAATTTTTTCCGATATGCTGAAAACCAAAGAACGTTTAGGA

Figure 8 (continued):

ATGACTAACTTTCAACAGAAAATTGAAGTGAAAATGGCTCAACATCCATTGCCAA
CACTGATTATGTGGGGCAAAGAAGATCGCGTTCTTGACGTATCCGCAGCAGCGGC
CTTCAAAAAAATAATTCCACAAGCAACTGTTTCATATTTTTCTGAAAGTAGGCCAC
CTACCTATGGTAGAAATTCCTAGTGAAAGCGCTAAAGTTTATGAAGAGTTTTTGT
CCTCTATTAAATAAGAGCACATAATCATGACTGACTTATAAACAGCCAAGCATTT
AAAATGCTTGGCTGTTTATTTTAATGGCCAAATTATTCAACGACCAAGCTCTGCG
GTAAAATCGCAGTGGGTTTCTTGTTTTTCATCAACAGCAACAAACGTGAAATACCC
CGTAATCGCATTTTTCTGATTATCAAAATACATACTTTCCACCAGCATATTAAC TT
CAACTTTTAAACTCGTCCGCCCTACCTCTATAACACTGGCAGTCAATTCGACAATG
GTACCTGCGGGAACAGGATGCTTAAAATCGATTTCGATCACTGCTGACGGTTACGA
TGCTTTGTGAGAAAAACGAGTCGCTGCAATAAAAGAAACCTCATCCATCCACTG
CATTGCAGTGCCACCGAATAACGTATCATGATGATTGTGTCTCTGGAAATACC
GCTTTAGAAATAGTGGTTTTTGATACGCGCTTTCGCTGCGCAATAATATCTTCTCT
GCTAAGAGTTGCGGATGGCATACTAACTCGCTTGATTAAAGATTAATAATAAAT
AGTTAACAGTATATTGAACTGAGGGTCTGAAGAACTCTAATACCTCTGAAGAACT
TTGAGGCCGCTAGAGAGAAAAGACCAGTGATAATATTTTCATCTTGCCATGAGAGC
TTATCATGAAAGCCTGTGCTTAAAATCAATCATTATATTTATTCATCTTTAATTGA
AATAATACCAATATATTTCATATATAATTTACACTACCCTTATCTCACTAGACTT
CCCGCGCATAGGCGCAAACAATCAACGCAAGTTCACAATAAAAGCGGTTCCGCTGC
AACACATGCCCTAGCGTCTAAAGTAGCACGCACAACACTGGCCAGTCGTACTAGC
CCCTTTGCGATTTCGTGCAGACGAGCAACAAGCGCTATTAAACTTACCTAAATTTCT
TAACCACCACCATTGGTTCTTTTCCACAAACTCAAAAAACTCGTCAAATCCGCTTG
CAATTTAAACGCGATGACATAGATCTAATCGATTATCAAACCCGCATTCAAGCGC
TCATTTAAAAACGCACCACTGGCAAGAAGTTCTACCTGCACTGACCAATATGCAAG
CGGCGGCGGAAGAGCTGCCFTTGATCGATCAAGAAGAAGGGAGCAGCAAAGAGG
AAAACAATCAAAAAGAGGAGAGCAATCAAATAAAAACGAGTTATTGAGGATTTT
AATTTTAAACAGGTATATTAATACCCTCTCTCGTAGTAAACAATGACTGTATTTA
CACAAAAATAAATAGAGGTATACCATGTCAAACATCTGGTTTGAAGTACCAAAG
ATTGAAGTATTAAACCGTCAAATGGAAAATACTGCCTGCAGCAACTTAGGCATTCT
AAATTACAGAAATTGGCGATGATTATATCACTGGCACAAATGCCAGCAGATGCACG
TACCTTCCAGCCAATGGGACTGATTTCATGGCGGCTCAAATGTATTGCTGGCAGAA
ACACTGGGCAGCATGGCAGCTAACTGCTGTATTAAATTTGTCTCAAGAATATTGTG

Figure 8 (continued):

TTGGCCAAGAAATTAACGCCAACCACATACGCGGTGTTTCGTTCCGGCATAGTGAC
TGGCACAGCAACGCTAGTACACAAAGGAAGAACCTCCCAGATTTGGGAAATTCG
CATCGTTAACGATCCAAAGAATTCAAAAGCTTCTCGAGAGTACTTCTAGAGCGGCCG
CGGGCCCATCGATTTTCCACCCGGGTGGGGTACCAGGTAAGTGTACCCAATTCGCCCT
ATAGTGAGTCGTATTACAATTCACCTGGCCGTCGTTTTAC

Figure 9:

Amino acid sequence of the stabilized single ring mutant Glu460Ala/Ser462Ala/Val463Ala of Cpn60:

SEQ ID No 14: Cpn10 (nucleotides 458-751 of Figure 10):

MKIRPLHDRVVRRKEEETATAGGHLPGAAAEKPNQGVVISVGTGRILDNGSVQALA
VNEG DVVVF GKYS GQNTIDIDGEELLILNESDIYGVLEA

SEQ ID No 15: Cpn60 (nucleotides 458-751 of Figure 10):

MAAKDVLFGDSARAKMLVGVN ILADAVRVT LGPKGRNVVIEKSFGAPIITKDGVSV
AREIELKDKFENMGAQMVKEVASQANDQAGDGT TTTATVLAQAIHSEGLKSVAAGMN
PMDLKR GIDKATAAVVAAIKEQAQPCLDTKAIAQVGTISANA DETVGR LIAEAMEKV
GKEGVITVEEGKGLEDEL DVVEGMQFDRGYLSPYFINNQEKMTVEMENPLILLVDKK
IDNLQELLPILENVAKSGRPLLIVAEDVEGQALATLVVNNLRGTFKVA AVKAPGFGD
RRKAMLQDLAILTG GQVISEELGMSLETADPSSLG TASKVVIDKENTVIVDGAGTEAS
VNTRVDQIRAEIESSTSDYDIEKLQERVAKLAGGVAVIKVGAGSEMEMKEKKDRVD
DALHATRAAVEEGV VAGGGVALIRALSSVT VVGDNEDQNVGIALALRAMEAPIRQI
AGNAGAAGAAVVDKVKSGTGSFGFNASTGEYGD MIAMGILDPAKVTRSSLQAAASI
AGLMITTEAMVADAPVEEGAGGMPDMGGMGGMGGMGMPGMM

Figure 10:

SEQ ID No 16: DNA sequence of the stabilized single ring mutant

Glu460Ala/Ser462Ala/Val463Ala:

In the DNA sequence:

Small letters – the Cpn10-Cpn60 coding fragment,

Big bold letters = introduced mutations

atcaaaaaatgcagcaaggacagattcctgcccagaafiagcagaaggffcttctgttagcactggccggcgcttlaftaltaacgccgg
gtttgtcactgatgcgtgggtttacattactgiccccgacgcgtaaaagcgttggccataagggtattgcatttattacccctc
gcatgatgactgcaagcagcittcaagcgacgggtagtttcaggaaggctcgittaaagatgtacatcgcacacigactcgcaagca
gtcatgaaaaaatcacaattgaaggcgaaatataccaaagacgataagtaggtatttttggctagccgttgaatctagtaaaagccc
cgafaaaitaaccatctattttcacagaggcaatttagccittgtttaccitattgafectaatcttgggatccaacagttggagagictagc
aaatgaaaatccgtccattacatgatcgtattgttgcgcgtaaaagaagagaccgcaactgcgggtgggtattatttacc
ggcgctgcggcagaaaaacaaatcaaggtgtgttactctgtgggtactggccgtattcttgataatggttcagtgcaagcgctggc
ggtaacgaaggcgatgtgtcgttttggtaatactcaggtcaaaatactatcgatacgtatgggtgaagaattaitgatttgaatga
aagtgtatctacggcggtttagaagcttaattattacactcacitttttatttaacctacaaaaittaaggaaagatcatggctgttaaagacg
tattatttgggtgatagcgacgcgcaaaaatgttggtaggtgtaaacatttagccgacgcagtaagagttaccttaggacctaa
aggctgtaacgttgttatagaaaaatcatttgggtgcacgcacatcaccaaaagatgggtttctgttgcgcgtgaaatcgaattgaagaca
aatcgaataacatgggcgcacagatggtaagggaagttgcttcaagccaacgaccaagccgggtgacgggcacaacgacagcgact
gtactagcacaggcgattatcagcgaaggcttgaatctgttgcggctggcatgaatccaatggatctfaaagctgglattgataaagcta
cggtctgtgttgcgccaitaaagaacaagctcagccttgcgttgatacaaaaagcaatgcctcaggtaggggacaatctctgccaatg
ccgatgaaacgggttggtcgtttaattgtgaagcgatggaaaagtcggtaaaaggggtgattaccgttgaagaaggcaaggcctt
gaagacgagcttgatgtttagaaggcatgcagttcgatcgcggtactgtctccgtacttcaacaacaacgaagaaaaatgaccgta
gaaatggaaaatcattatctatgtgttgataagaaaatgataacctcaagagctgttgcgaattcttgaaaacgfcgctaaatcaggt
cgctcattattgatcgttctgaagatgttgaaggccaagcactagcaacattggtagtaacaacttgcgcggcacattcaaggttgc
agcgggtiaaagccccctgggttggcgatcgtcgtaaagcgatgttgcgaatcttgcacatcttgacgggttggtcaggttattctgaagag
ctagggatgtcttagaaactcgggactcttcttgggtacggccaagcaagggtgttatcgataaagaaaacaccgtgattgttga
tggcgcagggtactgaagcaagcgtaataactcgtgttgaccagatccgtgtgaaatcgaaagctcgactctgattacgacatcgaaaa
gttacaagaacgggttgtaagcttgcggcgcggttgcgigaltiaagggttgggtcgggttctgaaatggaaatgaagagagaaa
gaccgtgttgacgatgcactcatgcaactcgcgcagcggtgaagaagggtgttcttgcgggtgggtgtgttcttctgattcgcgcactct
cttcagtaaccgtgtgttgataacgaagatcaaaacgtcggtattgcattggcacttctgtcgatggaagctctctacgcgtcaaatcgcc

Figure 10 (continued):

gggtaacgcaggtagctgCagggGcagCgggtgtgataaagtgaatctggcacaggtagcttgggttaacgccagcacaggtag
agtatggcgatatgatlgcgatgggtatfittagaccctgcaaaagtcacgcgttcattctcaagccgcggcgctatcgcaggttgat
gatcacaaccgaagccatgggtgcccgtggaagaaggcgctgggtgatgctgatatgggcggcattgggtggaatggg
cgggatgcccggcatgatgtaalcacttggatgcaatgtcctgatctgctaccgtgtaaaaagatcaggctcaaggctgtctctataaaa
agccglatcttgaatgagtggtgtctctctgctgaaaacgacattctggagtgccgcttttttgatttggcataaaaatcagaatattgtga
atttatgtaactagctggccataatgttgagtctctgggtggcatgatctcattggaacttcaacttaagccgattcaactgcg
gttitaacagtaaaataataacgcaacgtagaaacataataagcgatggcattaatgaagacggctgcatlfaattcagatc